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E. coli extract for bead halo assay

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An abbreviated version of this protocol was published in eLIFE in Jan 2017

Extensive cargo identification reveals distinct biological roles of the 12 importin pathways

DOI: 10.7554/eLife.21184

Detailed protocol

***E. coli* extract for bead halo assay**

Buffers:
STE: 10 mM Tris-HCl (pH 7.5, 4°C), 100 mM NaCl, and 1 mM EDTA.
Extraction buffer: 50 mM Tris-HCl (pH 8.0, 4°C), 500 mM NaCl, 1 mM EDTA, 10 mM 2-mercaptoethanol, and 0.5 mM PMSF.
Transport buffer: 20 mM HEPES-KOH (pH 7.3), 110 mM KOAc, 2 mM MgOAc, 5 mM NaOAc, 0.5 mM EGTA, 2 mM DTT, and 1 µg/mL each of aprotinin, pepstatin A, and leupeptin.

Strain: BL21.
Vector: pQE80L (Qiagen).
Culture: 15 mL LB (Amp) at 37°C till OD_{600}=0.4
Induction: when OD_{600}=0.4, shift to 20°C, add 0.1 mM IPTG, and incubate at 20°C for 16 hr.
Harvest: chill the culture on ice, spin down the cells at 4°C, and remove supernatant. Suspend the cells in 1 mL ice-cold STE, spin down at 4°C, and remove the supernatant. Freeze the cell pellet in liquid N_2 and sore at -80°C.

Extraction:
Suspend the frozen cells in 0.3 mL ice-cold Extraction buffer containing 0.3 mg/mL lysozyme, and stand on ice for 20 min. Sonicate on ice using a small tip for 10 sec ×2 times (we use Handy Sonic UR-20P (TOMY) at power level “3”). Centrifuge at 20,000 g for 15 min at 4°C. Recover the supernatant and dialyze it against Transport buffer at 4°C for >6 hr. Centrifuge at 20,000 g for 15 min at 4°C. Recover the supernatant, aliquot, freeze in liquid N_2, and store at -80°C until use.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Kimura, M. and Imamoto, N. (2021). E. coli extract for bead halo assay. Bio-protocol Preprint. bio-protocol.org/prep1242.
2. Kimura, M., Morinaka, Y., Imai, K., Kose, S., Horton, P. and Imamoto, N.(2017). Extensive cargo identification reveals distinct biological roles of the 12 importin pathways. eLIFE. DOI: 10.7554/eLife.21184